R&D of Long-Term Life Support System by Using Electrochemically Activated Biofilm Reactor of Aquatic Animals for Space Examinations

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Abstract We have developed the long-term life support system that enables the experiment of aquatic animals breeding for 90 days or more for the future experiments in orbit. In order to enable long-term breeding of wide aquatic animals, it is necessary to remove nitrate produced by biological nitrification. Then, we examined a denitrification method to use an electrochemical reaction of biofilm-electrode reactor. In this research, we have not kept the aquatic animals actually but imitated breeding of five goldfish. The ammonia of about 250 ppm was added in breeding water through 90 days. As a result, neither ammonia nor nitrite accumulated 0.1 ppm and nitrate could be suppressed to about 10 ppm.

Key words; electrochemical denitrification, long-term life support system, biofilm, biofilm-electrode reactor

Introduction

We have developed the long-term life support system that enables the experiment of aquatic animals breeding for 90 days or more for the future experiments in orbit. As for breeding of aquatic animals under the space environment, the effective use of limited water resource becomes a problem (Endo *et al.*, 1999). It is thought that breeding aquatic animals without exchanging the water in the closed system is important at the point of saving the water resource and researching the material circulation under closed environment (Endo *et al.*, 1999). Many experiments with various aquatic animals have been performed in the space shuttle (Ijiri, 1995; Nagaoka *et al.*, 1998) and those aquatic animals have been bred in sealed containers with a limited amount of water under microgravity. The development of basic technology for the life support system is indispensable for biological research in space (Nakamura *et al.*, 1996a; 1996b; Nakamura *et al.*, 1998a; 1998b).

Both the stable removals of ammonia and nitrite and countermeasures against decrease of pH in the breeding water are necessary for aquatic animals breeding (Nakamura *et al.*, 1996a). Biological nitrification is a very effective way to resolve this problem (Nakamura *et al.*, 1996b). Biological nitrification is the reaction of ammonia and/or nitrite oxidizing to nitrate by two kinds of nitrifying bacteria. In earlier studies, it is not necessary to remove nitrate promptly because of the low toxicity. However, in order to enable long-term breeding of wide aquatic animals,

it is necessary to remove nitrate in the water, because the accumulation of nitrate causes the pH to decrease.

Biological denitrification, which is one of the effective ways to remove the nitrate in the water by reduction of nitrate or nitrite to nitrogen gas, required organic compounds as an electron donor (Knowles, 1982). The availability of electrons in organic compounds is one of the most important factors controlling the activity of biological denitrification. Methanol is used as organic compounds in general waste water treatment (Suzuki *et al.*, 1993). However, it cannot be used for space experiments, because it is combustible and toxic for aquatic animals. We applied the method of the denitrification used glucose as organic compounds, and succeeded in breeding on goldfish (fresh-water fish) and oyster toadfish (marine fish) for 90 days (Nakamura *et al.*, 1998a; 1998b).

In this research, we adopted the electrochemical reaction of biofilm-electrode reactor (Sakakibara *et al.*, 1993-1997) instead of adding organic compounds for biological denitrification, and purified the breeding water. In this paper we described the results of the experiment of purifying breeding water with the life support system supplemented the biofilm-electrode reactor.

Material and method

Experiment apparatus

The flowsheet of the experiment apparatus is shown in Figure 1 and Table 1 shows the specification. The total volume of breeding water was 3.2 L and flow rate was assumed to be 1 L/min. The gas exchange (oxygen/carbon dioxide) in the breeding water was with the pump oxygenator (Terumo Capiox II 08). The breeding water temperature was controlled 23 °C. We loaded 1.8 mg/L of ammonia to imitate five goldfish (body length about 3 cm, body weight about 1.5 g) breeding, and fed 0.2 g of food

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Table 1 Specification of the Experimental Unit

Water	distilled water
Water volume	3.2 L
Operation temperature	23°C
Circulation rate	1 L/min
Feeding	0.2 g/day
Nitrification tank volume	400 ml (glass beads)
Denitrification tank volume	1000 ml (carbon pellet)
Experimental period	90 days

(Kyorin Co., Ltd., Babygold) everyday. The water purification system consists of combined biological method (nitrification and denitrification). 400 ml of nitrifying bacteria adhesion glass beads carrier (Schott, Siporax) was filled in the nitrification tank and 1000 ml of denitrifying bacteria adhesion carbon pellets (Koujundokagaku-kenkyujo Co., Ltd.) was in the denitrification tank. The nitrifying bacteria we used here are the same strains in the Vestibular Function Experiment Unit (VFEU) at the NEUROLAB and STS-95 missions (Nagaoka *et al.*, 1998). We used two kind of strains, one was *Nitrosomonas* sp. MK8 (FERM P-12555) and another was *Nitrobacter* sp. IM203 (FERM P-12556) as nitrifying bacteria (Nakamura *et al.*, 1991). Electric current value running through the electrodes in the denitrification tank was controlled to be 5mA, and retention time of denitrification tank was assumed 6 hours.

Water analysis

The concentrations of ammonium (NH₄-N, indophenol blue photometry), nitrite (NO₂-N, Griess-Romijn Method), nitrate (NO₃-N, brucine sulfanilic acid method) and pH (pH electrode, COS) were measured.

Results

The relation between electric current and denitrification rate is shown in Figure 2. We defined a denitrification rate

Fig.2 Electric current-dependence of denitrification rate **Fig.1** Diagram of experimental apparatus.

as a nitrate decrease of one liter of denitrification tank water at a day. The denitrification rate was a linear function of electric current in the range of 1-5mA. The excretion of five goldfish was calculated about 5.8 mg/d of ammonia and about 5mg of ammonia was included in 0.2g of fish food “Babygold”. Thus, when five goldfish are bred in this system, about 11mg of ammonia is accumulated in breeding water everyday. As the denitrification tank keeps 500 ml of water, denitrification rate required more than 22 mg/L/d to prevent nitrate accumulation. As was clear from Fig. 2, it was found that the required denitrification rate (22 mg/L/d) was obtained in running at 2mA or more. On the other hand, it was 4mA that theoretical electric current value to obtain the required rate above. We decided the electric current value at the long-term examination 5mA, because of processing performance change and the theoretical electric current value.

Figure 3 shows time course of NH₄-N, NO₂-N and NO₃-N concentrations and pH of breeding water on the long-term examination. Ammonia and nitrite were continuously 1ppm or less through the examination period. Nitrate remains little until the 30th day and has increased up to about 10ppm after that. However, after the day 30, nitrate concentration became constant about 10ppm and pH

Fig.3 Time course of water quality

indicated stable about 8.

Discussion

Since 1992, we have achieved five experiments with aquatic animals on space shuttle missions, which were named FMPT, SL-J, IML-2, NEUROLAB and STS-95. These previous experimental periods were short term, up to 3 weeks. The purification method in these experimental units was chemical adsorption with zeolite or biological nitrification. However, more long experimental period will be required for the future experiments in orbit such as those at the International Space Station (ISS). Aquatic animals can be expected to be kept for 90 days or more in space. Though nitrate was not removed at the shuttle experiments, it will be necessary to remove nitrate in the breeding water at the ISS experiments. It is because high accumulation of nitrate caused the toxicity to increase and the pH to decrease.

Biological denitrification is one of nitrate removal methods. We had already applied this method for life support system and succeeded in breeding on goldfish and oyster toadfish for at least 90 days (Nakamura *et al.*, 1998a; 1998b). However, this system required excess carbon source (i.e. glucose), and that we added excess glucose in our previous experiment (Nakamura *et al.*, 1998a; 1998b). The result was that carbon compounds in breeding water increased and various microorganisms grew to consume the carbon compounds in breeding water. Therefore, we desired new water purification method by which nitrate was removed and no pollution of breeding water occurred besides.

In this research, we used the biofilm-electrode reactor for denitrification and obtained satisfactory results, that is, no accumulation of nitrate and pH maintenance of comfortable range for aquatic animals as shown in fig 3. These results demonstrated the effectiveness of using life support system supplemented the biofilm-electrode reactor.

The purpose of this study was to obtain running data of water quality, when constant ammonia loading was given by addition of chemicals instead of breeding aquatic animals actually. The performance under the condition of irregular loading will be obtained by breeding aquatic animals actually in the future work. Moreover, we are also scheduled to examine processing of the bubbles of nitrogen gas generated by denitrification reaction.

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